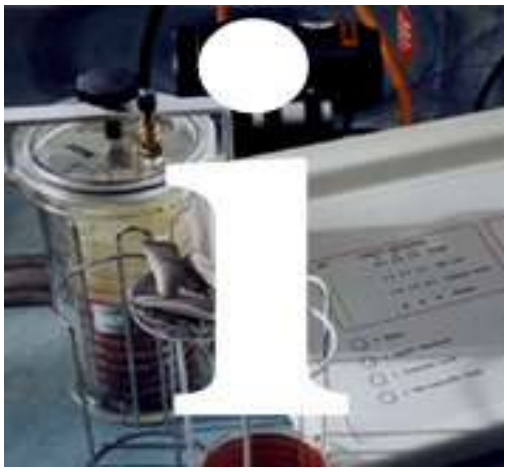
MART Microbiology BV

User Manual

Anoxomat

Systems



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User Manual Anoxomat

Introduction

The MART® Anoxomat® system is an automated system which creates an anaerobic, micro-aerophilic or capnophilic atmosphere in an anaerobic jar, just by a push on the button and in about one minute time. Evacuating the jar and filling it again with an oxygen free gas mixture, according to the method of MacIntosh and Fildes, create these atmospheres.

The MART® Anoxomat® system controls the process and performs a quality assurance test to ensure that all conditions for optimum bacterial growth are realized.

This manual is meant for users of the MART® Anoxomat® system models WS80, WS8000, WS8080 and WS9000.

We are happy to inform the user about the general characteristics and the specifications of the system, the installation and the principle of operation are also described.
The principles of programming the WS8000, WS8080 and WS9000 are described in the paragraph about the programming panel.

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Configuration of the Anoxomat system

Anoxomat system

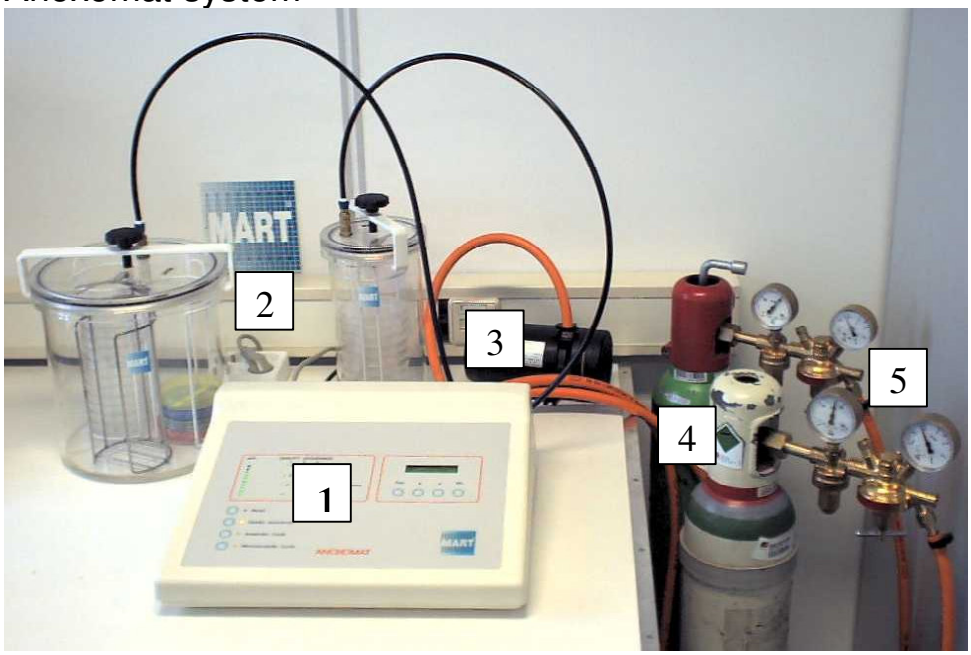


Figure 1: Anoxomat system WS8080

1. Anoxomat WS-Model
2. Mart Jars
3. Vacuum pump
4. Gases
5. Reducing valves (1.8 bar)

Depending on the model 1 up to 3 gas cylinders may be used, and 1-upto 3 jars may be used

Specifications

Anoxomat characteristics

Mart Microbiology B.V. produces 4 different systems, for specific use. All models incorporate the complete quality assurance programs and the standard basic Micro-aerophilic and Anaerobic recipe.

Table 1: Characteristics

Characteristic / model	WS80	WS8000	WS8080	WS9000
Jar connections	1	3	2	1
Gas connection 1 [1]	Mix [2]	Mix [2]	Mix [2]	Mix [2]
Gas connection 2 [1]	--	--	CO_2	CO_2
Gas connection 3 [1]	--	--	--	N_2
Quality Assurance Program[3]	Yes	Yes	Yes	Yes
Gas pressure	1.8 bar	1.8 bar	1.8 bar	1.8 bar
Standard programs	Micro-aerophilic Anaerobic	Micro-aerophilic Anaerobic	Micro-aerophilic Anaerobic	Micro-aerophilic Anaerobic
Programming features	--	yes	Yes	yes

[1] The gas connections of the Anoxomat are non-specific, i.e. it does not matter what kind of gasses are connected to the system.
In the Mart Microbiology B.V. manual, gas supply 1 is always the anaerobic gas mixture, gas supply 2 is CO_2 gas and gas supply 3 is N_2 gas.

[2] Anaerobic gas mixture:
80 to 90% N_2
5 to 10% CO_2
5 to 10% H_2.

[3] The Mart Anoxomat quality assurance system consists of:
Jar leak test
Gas input and pressure test
Catalyst activity test
Extended Jar leak test

Anoxomat technical specifications

Table 2: Electrical specification

Parameter	Value	Value
Voltage	220-230 V (see rear plate)	110-120 V (see rear plate)
Frequency	50 to 60 Hz	50 to 60 Hz
Fuse 1	160 mA slow	315 mA slow
Fuse 2 -3	6.3 A slow	6.3 A slow

Reducing valve

Table 3: Technical requirements of the reducing valve

Max. in pressure	200 bar
Range	0 ..4 bar
Capacity	Max. 5 nm^3 / h
Inlet	NEN 3268
Outlet 1	G 3/8" left turning helix, anaerobic gas
Outlet 2	G 3/8" right turning helix, CO_2
Outlet 3	G 3/8" right turning helix, N_2
Weight	2.3 kg

Gas

The standard gas mixture used for anaerobic bacteria cultivation as well as for micro-aerophilic bacteria cultivation, is made of:

Table 4: Mixgas composition

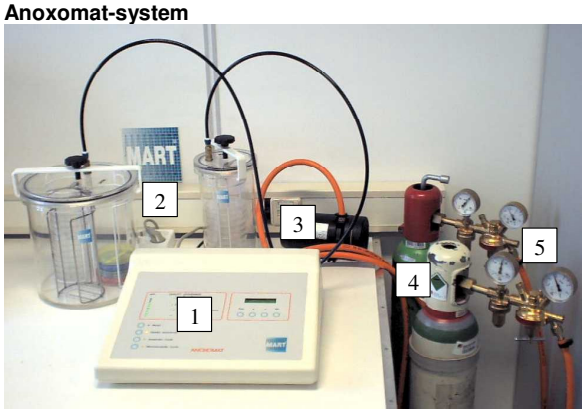
N_2	**Nitrogen**	**80 to 85%**	**(anaerobic basic gas)**
CO_2	Carbon dioxide	5 to 10%	(growth stimulation)
H_2	Hidrogen	5.. to 10%	(catalysis)

Installation

For details of installation, please consult the instruction card hereafter which is also delivered separately with the machine. This card describes the installation of the MART® Anoxomat® system in detail. Installing the Anoxomat system is only a matter of minutes when all components are available.

MART® ANOXOMAT – SYSTEM
INSTRUCTION - CARD

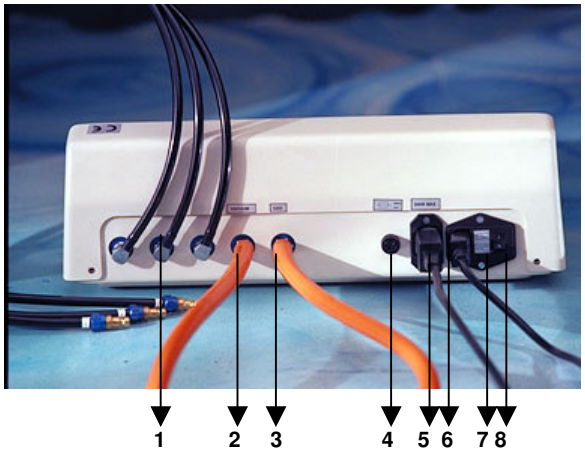
Anoxomat-system



1. Anoxomat WS-Model
2. Mart Jars
3. Vacuum pump
4. Gases
5. Reducing-valves (1.8 bar)

Depending on the model 1-upto 3 gas cylinders may be used, and 1-upto 3 jars may be used

Connections

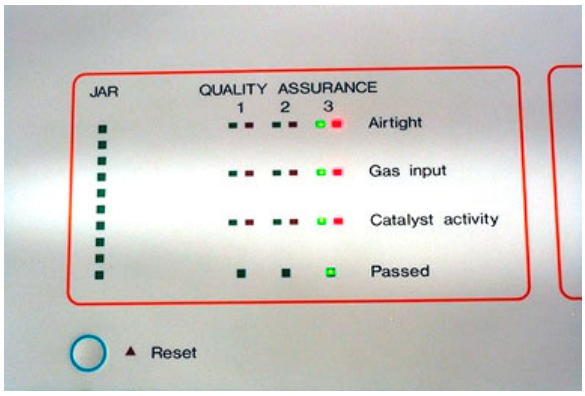


1) Jar-tube(s) (up to 3 Jars)
   a) Inside diameter 6 mm.
2) Connection vacuum pump ("Vacuum")
   a) Inside diameter 8 mm.
   b) Length max. 2 m.
3) Connection gas cylinder (up to 3 gasses. “Gas”)
   a) Inside diameter 8 mm.
   b) Length max. 2 m.
4) Fuse 160mA slow, version 220V
   Fuse 315mA slow, version 110V
5) Power-supply vacuum pump ("PUMP")
6) Power- supply 220 / 110 Volt ("POWER")
7) 2 x fuses inside (2 x 6.3 A)
8) On/Off switch

Installation

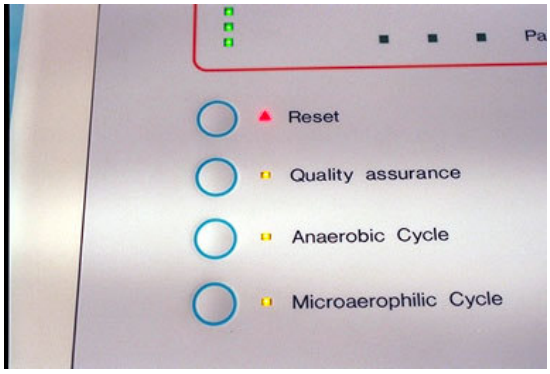
1) Connect the Anoxomat to a socket.
2) Plug the power cord of the vacuum pump in the back of the Anoxomat.
3) Push on the tubes between:
   a) the Anoxomat and the vacuum pump.
   b) the Anoxomat and the gas cylinder.
   Fasten the tubes with the tube clamps
4) Open the gas supply on the gas cylinder
5) Set the gas-pressure with the reducing-valve on 1.8 bar **precisely**.
6) Switch on the Anoxomat.
7) After about 10-15 seconds the red and yellow LED’s on the Anoxomat switch out (= Start-up time).
8) The Anoxomat is ready for use.
9) Connect one or more jars with the jars-tubes
   a) Pull the outer ring of the valve on the jar down and push-in the black jar-tube.
   b) To disconnect hold the jar-tube and pull down the outer ring of the jar-valve.

Front-Panel



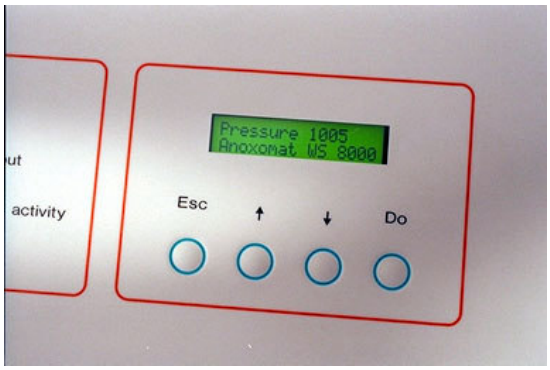
1) **Jar**
   a) LED’s monitoring system-pressure
      i) Every green LED is 100 mbar
   b) Quality assurance level
      i) LED 1 is level one, etc.
2) **Quality assurance**
   1 2 3
      i) Jar indication
      ii) Gas supply indication
   a) Airtight
      i) Jar 1, 2 or 3
      ii) Indication if the anaerobic jar is airtight or not
      iii) green = airtight, red = not airtight
   b) Gas input
      i) Gas 1, 2 or 3
      ii) Indication if gas-pressure is right
      iii) green = right, red = to much or not enough
   c) Catalyst activity
      i) Jar 1, 2 or 3
      ii) Indication if catalyst-activity is sufficient
      iii) green = sufficient, red = not sufficient
   d) Passed
      i) Jar 1, 2 or 3
      ii) green = passed, red = failure

Programs



1. **Reset**
   a) Reset program and errors
   b) Standard settings are kept
2) **Quality Assurance**
   a) To engage, press 1- to10 times before the anaerobic or micro-aerophilic program is started. (“Jar” LED’s light up accordingly.)
   b) Following tests are performed:
   c) Level 1 (bottom LED lights up) : Jar vacuum-leak test + gas-input test.
   d) Level 2: Jar vacuum-leak test + gas-input test + catalyst test (**Standard level** 180 sec.)
   e) Level 3: Jar vacuum-leak test + gas-input test + catalyst test (75 sec.)
   f) Every higher level: 30-sec. longer catalyst test.
3) **Anaerobic cycle**
   a) Choose the required Quality Assurance level (view point 2)
   b) 3 Evacuations and refills to atmospheric pressure
   c) With Catalyst the oxygen level will be **0%**
4) **Microaerophilic cycle**
   a) Choose the required Quality Assurance level (view point 2)
   b) 1 Evacuation and refill to atmospheric pressure
   c) Oxygen level 6%

Programming-facilities



The Anoxomat-models WS8000, WS8080 and WS9000 are provided with a programming-panel and a display, to allow the user to program up to 40 recipes

1) **Display**
   a) Atmospheric pressure
   b) System-pressure during operation
2) **Buttons**
   a) [**Esc**]
      i) Switch from operation to programming mode
   b) [↑↓]
      i) step up or down for menu-level
      ii) not all levels appear by stepping down
   c) [**Do**]
      i) Confirm selection
      ii) Run recipe

Trouble shooting

1) No start up
   a) No electricity
   b) Fuses are broken
   c) System-error
2) A red LED lights up at [**Reset**]
   a) Remaining vacuum in the jar
      i) On digital display "closed" (not at WS80)
   b) Vacuum pump not switched on or connected
   c) Vacuum pump defect
   d) Gas cylinder closed
   e) Jar-capacity more than 8 dm^3
3) Jar leak [**Airtight**]:
   a) Jar not airtight
   b) Lid not airtight
   c) Jar coupling not airtight
   d) O-ring not sealing off
   e) Stainless steel jar clamp not turned tight
4) A red led lights up at [**Gas input**]:
   a) Gas pressure to high or to low
      i) Check reducing valve on gas cylinder
   b) Gas cylinder closed
      i) Check reducing valve on gas cylinder
   c) Leak in gas-tube or at connections
   d) No gas connected
5) A red LED lights up at [**Catalyst activity**]:
   a) No Catalyst used with QA-level 2 and up
   b) Catalyst used with Microaerophilic cycle
   c) Bad quality of catalyst
      i) reactivate catalyst
      ii) replace catalyst
6) Atmospheric pressure on display < 800 mbar or > 1200 mbar
   a) not with WS80 (no display)
   b) Display reads “pressure failure”
7) No green led at [Passed]:
   a) Program-error occurred
   b) System-error occurred

User-Tips

1) Anoxomat:
   a) Always perform Jar-leak test (Quality Assurance)
   b) Reactivate catalyst regularly
      i) 0.5 - 1 hours at 160ºC - 100ºC
   c) Take care that no dust comes into the (unused) Jar-tube(s)
2) Mart Anaerobic Jar:
   a) Grease the O-ring regularly
   b) Grease the coupling 4 times per year with teflon spray
   c) Do not over-tighten stainless steel jar clamp
      i) TIGHT = TIGHT!
   d) Do not disinfect the Mart Anaerobic Jars with alcohol!
      i) Use disinfectant provided by Mart Microbiology B.V.
3) General
   a) Do not switch the Anoxomat on and off within a few seconds.
   b) Install the gas cylinder(s) according to local standards and regulations.

For further information, contact your local dealer.

Test the equipment

The gas supply is opened and by means of the reducing valve the operational pressure should be reduced to 1.8 bar.

Caution: Too much deviation from the required operational pressure may cause malfunctioning, or even damage the system.

After the electrical cables, the vacuum/gas tubes, the frequency (50-60 Hz) and voltage (220-230V resp. 110V) marked at the back are checked, the system can be switched on with the switch next to the power plug at the backside.

The Anoxomat self test program performs a test on the functioning of the individual Anoxomat system components, which appear to the user as follows:

- The vacuum pump inlet valve and the jar valves are opened and closed (clicking of the valves)
- The pump is tested. The pump will run a few seconds.
- The light emitting diodes (LED) on the indication panel illuminate.
- System is calibrating to the actual atmospheric pressure.
- An acoustic signal indicates that the Anoxomat is in the operational mode and the display (only WS8000, WS8080 and WS9000) shows the actual atmospheric pressure and the Anoxomat type number.

The Anoxomat self-test program takes about 15 seconds.

Caution: Do not switch off the system during the Anoxomat self test program.

Principle of operation

In this paragraph the principle of operation of the Anoxomat will be explained. The main system components of the Anoxomat are:

- User interface
- Processing unit
- Valve and pressure system
- Electronics to control the valve system and vacuum pump
- Electronics to control the display and sensor signals

At the user interface three different areas can be distinguished which will be described successively:

- Operator panel
- Indicator panel
- Programming panel (not on WS80)

Operator Panel

The user who uses the Anoxomat in daily laboratory routine connects one or more jars and selects the required program on the operator panel to start one of the standard recipes. After the program is completed (program ready signal sounds) the jars can be disconnected and stored into an incubator.

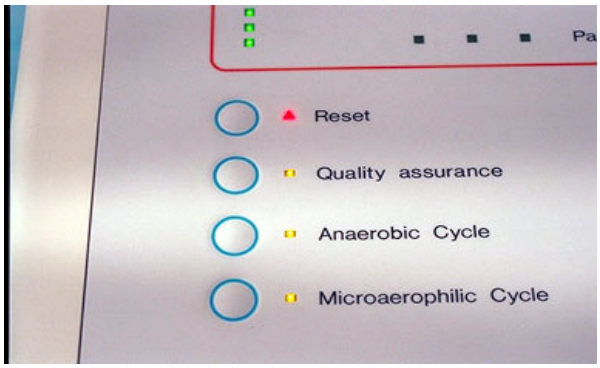


Figure 2: Operator Panel

A yellow light emitting diode on the right side of the push button illuminates if the user pushes the button on the operator panel.

Another possibility is to start a (user-defined) recipe by pushing the DO button on the programming panel. The actual selected recipe is executed. How to change the actual recipe is described in the paragraph "Selection of a recipe" in this manual.
Activation of the recipe with the DO button instead of the standard anaerobic or micro-aerophilic button is done when there are specific requirements for the jar atmosphere.

In subsequent paragraphs the following operator panel functions will be described briefly:

- Reset the Anoxomat,
- Start standard micro-aerophilic recipe,
- Start standard anaerobic recipe,
- Select the quality assurance level.

Reset

The main purpose of the reset button is to prevent errors. Users are made aware of erroneous situations and are forced to take the action required. Before another jar can be processed the reset button has to be pushed first. A signal sounds when other switches are pushed.

Standard Micro-aerophilic recipe

The standard micro-aerophilic recipe has one evacuation and replacement cycle, which is composed of two phases:

- evacuation phase
- replacement phase

The standard micro-aerophilic recipe composes optimum breeding results for micro-aerophilic organisms, such as for instance *Campylobacter jejuni*.

For WS80 and WS8000 users, it could be of interest that J.S Brazier[2] proved good growth conditions for capnophilic bacteria (*Neisseria, Heamophilus en Gardnerella*) in the jars, which were processed with the standard micro-aerophilic recipe. The users of the WS8080 and WS9000 have the possibility to create micro-aerophilic atmospheres in a jar, with various O_2, CO_2, and N_2 concentrations.

Calculation rest percentage oxygen in standard micro-aerophilic recipe

In the first evacuation phase of the standard micro-aerophilic recipe of all Anoxomat models, 70% of the jar volume is evacuated. The Anoxomat achieves this with high accuracy, due to the high precision pressure sensor. Assuming the atmospheric pressure is 1030 mbar; the standard micro-aerophilic recipe evacuates until 0.3 * 1030 = 309 mbar.

In the successive replacement phase, the jar is filled with an oxygen free gas mixture.

Initially there is approximately 20% oxygen in the atmosphere in the jar, the same percentage as normally in the air. At the end of the evacuation cycle only 30% of the original gas volume in the jar is left. There is no oxygen in the replacing gas mixture, so after the replacement cycle there is:
0.3 x 20% = 6% O_2 in the jar.

With the programmable Anoxomat models, recipes for a reduced oxygen concentration atmosphere can be programmed easily as described in the paragraph "Programming recipes". A similar calculation is required before programming.

Assume that a reduced oxygen atmosphere with 8% oxygen is required. To obtain this atmosphere, (8/20) 40% of the oxygen must remain in the jar, so 60% of the jar volume should be evacuated. The necessary low set point for this recipe can be calculated directly and entered into the program. (1030*0.4=set point 412mbar)

Standard anaerobic recipe

The standard anaerobic recipe performs three evacuation and replacement cycles, which are different from the one evacuation and replacement cycle of the micro-aerophilic recipe.
Instead of evacuating 70% of the jar volume, in the anaerobic evacuation phase 80% of the jar volume is evacuated. The standard anaerobic recipe repeats this evacuation and replacement cycle 3 times as is described in several scientific publications [2][3].

Initially there is approximately 20% oxygen in the atmosphere in the jar, the same percentage as normally in the air. After every evacuation cycle only 20% of the original volume in the jar is left. There is no O_2 in the replaced gas mixture, so after the three replacement cycles there is: $(0.2)^3$ x 20% = 0,16% O_2 in the jar.

The catalyst takes this very small percentage of oxygen remaining in the jar away, which should be included in the jar with the anaerobic cycle.

Quality assurance

The integrated Quality Assurance program assures a desired jar atmosphere in advance.
In bacterial cultivation the key is that the cultivation environment is guaranteed and reproducible.
To assure this, the following primary aspects have to be certain:

- airtight jars
- appropriate gas supply
- sufficient catalyst activity

With the standard micro-aerophilic and anaerobic program, and with programmed recipes including a quality test, the quality assurance test is selected by pushing the "Quality assurance" button on the operator panel. The quality assurance is selected before one of the standard recipes is started.

Each time the button is pushed, the level is increased by one and one more light emitting diode on the LED bar will illuminate.

If the level is changed, this level is set until the system is switched off/on or a new level is selected. The level can be selected from level one up to level ten, indicated by the LED on the indicator panel

Table 5: Levels of quality assurance

Level (lit LED)	**Test**	**Test**	**Test**	**Time of catalyst test**
1	Jarleak test	Gasinput test		
2	**Jarleak test**	**Gasinput test**	**Catalyst test (incl. Extended leaktest)**	**(180Sec.)**
3	Jarleak test	Gasinput test	Catalyst test (incl. Extended leaktest)	(75Sec.)
4	Jarleak test	Gasinput test	Catalyst test (incl. Extended leaktest)	(105Sec.)
5	Jarleak test	Gasinput test	Catalyst test (incl. Extended leaktest)	(135Sec.)
6	Jarleak test	Gasinput test	Catalyst test (incl. Extended leaktest)	(165Sec.)
7	Jarleak test	Gasinput test	Catalyst test (incl. Extended leaktest)	(195Sec.)
8	Jarleak test	Gasinput test	Catalyst test (incl. Extended leaktest)	(225Sec.)
9	Jarleak test	Gasinput test	Catalyst test (incl. Extended leaktest)	(255Sec.)
10	Jarleak test	Gasinput test	Catalyst test (incl. Extended leaktest)	(285Sec.)

We always advise level 2.

The level of the quality assurance test is displayed on the indicator panel (see Figure 3). Under certain circumstances users might prefer to do a more or a less severe catalyst test. In case of new and reactivated catalysts the test may pass quicker than the selected test level, thus saving time. In case of older but reactivated catalysts a longer testing period may be necessary. It is necessary to order new catalysts!

In user defined (programmed) recipes the quality assurance functions can also be included. In this case the desired quality assurance level has to be activated as well on the operator panel before starting the user-defined recipe.

For including quality assurance functions in a user defined recipe see paragraph "programming recipes".

Indicator Panel

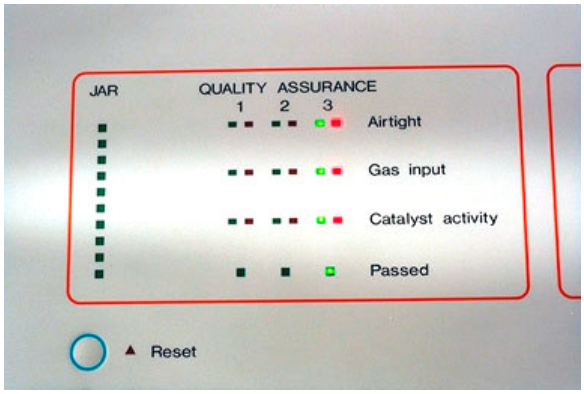


Figure 3: Indicator panel

Green light emitting diodes illuminate when all conditions are satisfied. In alarm situations the red light emitting diodes illuminate. When all quality assurance tests are passed the light emitting diode "Passed" will illuminate.

Light emitting diode bar “JAR”

The light emitting diode bar “JAR” has 2 main functions:

- Indicates the stage of the evacuation and replacement cycle.
- Indicates the level of quality assurance.

All light emitting diodes in the vertical row under the word "JAR” will illuminate when either the standard micro-aerophilic recipe or the standard anaerobic recipe is executed. When all light emitting diodes are on, the pressure in the jar(s) is atmospheric.
In the evacuation phase the volume is evacuated and the light emitting diodes extinguish one by one. The light emitting diode row indicator is a measure for the volume evacuated from the jar, i.e. if only one half of the light emitting diodes illuminate, 50% of the jar volume is evacuated.
In the replacement phase the light emitting diodes indicate that the anaerobic gas is injected.

Quality assurance light emitting diodes

The quality assurance light emitting diodes show the operator whether all conditions for successful bacterial cultivation are fulfilled or not.
The processing unit performs a range of tests to guarantee these conditions. The green light emitting diode "Passed" (Fig. 3) will illuminate when all quality assurance tests are passed successfully. When one of the conditions failed to pass one of the tests, the Anoxomat rejects the anaerobic jar, and a red light emitting diode will illuminate to indicate the failure cause.
The selected recipe will not continue with this jar but will proceed with the other jar(s). If the other jars are processed correctly, they can be disconnected and incubated.

The erroneous jar can be processed again after the Reset button on the operator panel is pushed. In this way the user is obliged to find the cause of failure. Accidentally continuation after failure is not possible.

The quality assurance tests are split into three groups:

- jar leak test,
- gas input test,
- positive or negative catalyst test.

In next paragraphs the quality assurance tests will be discussed in more detail.

Airtight

There are diverse types of leaks in jars:

- Very fine cracks, thin like a hair and hardly visual.
- A fixed particle on the seal between jar and lid,
- In case of intensive (improper) usage. A jar lid might not be fully smooth or the jar ring could be damaged, dry or cracked.
- Due to improper maintenance of the coupling on the jarlid, the coupling may not be airtight when the jar tube is disconnected

Some leaks only appear in case of overpressure and others only in case of underpressure. All kinds of leaks, even the very small ones, influence the reproducibility of bacterial cultivation, and are unacceptable.
The Anoxomat has a high precision pressure sensor and with a special algorithm, implemented in the processing unit's software, a wide range of leak types will be detected.
When the standard anaerobic or standard micro-aerophilic recipe is selected, no leak test is executed. However it is advised to run the quality assurance test regularly to test the jars extensively.
Many publishers emphasize the importance of the total absence of oxygen in relation to survival and growth of anaerobic bacteria, as is for example quoted in a reference [4] publication:

The rapid achievement and maintenance of a low oxygen tension, or the absence of oxygen, is an essential requirement for the successful cultivation of anaerobics.

For the WS8000 and WS8080 the test is applied successively for each connected jar and for the WS80 and WS9000 for one single jar. If one or more of the jars has a leak, the matching light emitting diode illuminates on the indication panel.

Gas input

In the second part of the quality assurance test, the availability of the replacement gas is tested.
A red LED will show if the pressure supplied by the reducing valve of the cylinder to the Anoxomat input is lower or higher than 1.8 Bar.
WS8000: LEDS 1, 2 and 3 will respond to the respective jar tubes.
WS8080: LED 1 responds to gas connection no. 1 and LED 2 responds to gas connection no. 2, only in case the 2 gasses are actually used. With standard recipes (anaerobic and microaerophilic) LED 2 will not show any response.
WS9000: LED 1 responds to gas connection no. 1, LED 2 responds to gas connection no. 2 and LED 3 responds to gas connection no. 3, only in case the 3 gasses are actually used. With standard recipes (anaerobic and micro-aerophilic) LED 2 and 3 will not show any response.

Catalyst activity

To create an anaerobic atmosphere, there have to be palladium coated aluminium pellets in a steel gauge sachet, in the clip of the jar lid, to serve as a catalyst. The MART® Catalyst is a cold catalyst. The function of the catalyst is to stimulate the reaction of H_2 with O_2 to H_2O.
Media, if they are not prereduced, contain oxygen, which will come in the jar atmosphere during incubation.

In the literature [5] the following statements about the catalyst are made:

- the pellets can be inactivated by excessive moisture and H_2S, therefore, they should be reactivated after each incubation by heating the sachet of pellets to 160°C in a drying oven for 1-1/2 to 2 hours.
- Reactivated catalysts should be stored in a dry area until used.

Compared to gas generating sachets the Anoxomat has:

- a lower moisture production in the jars
- less heat production in the catalyst

The catalyst should be renewed regularly because irreversible degradation of catalytic activity takes place (due to chemical binding with S-ions). The end-of-life period depends on the type of bacterium cultivated or, more accurate, the H_2S production. Therefore it cannot be indicated exactly how many incubation cycles can be executed with one sachet.

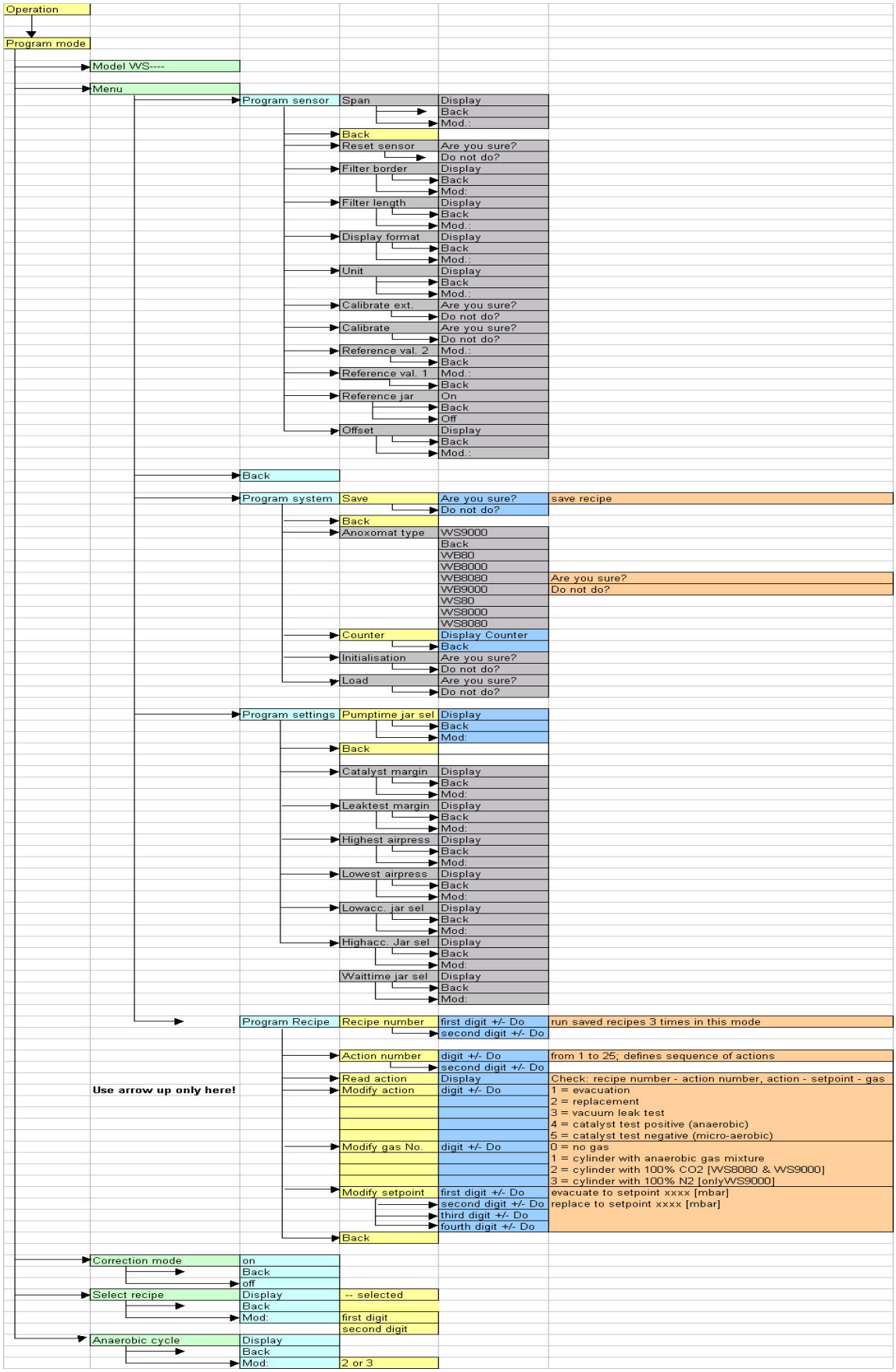
Programming panel

With the programming panel (Figure 4) all variable settings of the Anoxomat system can be changed.

Figure 4: Programming panel WS8000, WS8080 and WS9000.

With the programming panel a high flexibility of the Anoxomat is achieved.
Please find hereafter the menu structure used for the Anoxomat:

Figure 5: Programming tree



The Anoxomat systems with a programming panel have two modes:

the operational mode
and
the program mode.

With the Esc button you can switch from the program mode to the operational mode.
In the previous paragraphs the Anoxomat was assumed to be working in the operational mode.

Terms used for the program mode are:

menu
- list with items to select from.
- pushing the ↑ button displays the previous item (circular) and the ↓ the next item.

level
- each menu contains items on a certain level
- one item is always "Back", leading to the previous level.

item
- selection item (chapter), which is always on the display of the programming panel.
- by pushing the DO button an item is selected.
- with "Back" a level menu is selected for other items on a lower level.
- at the lowest level, DO assigns a parameter.

Changing the number of evacuation replacement cycles

The programming features can be demonstrated by for example changing the number of evacuation replacement cycles of the standard anaerobic program.

Table 6: experiment; change the cycles of the anaerobic recipe

Esc	Program mode
↑,↓	Until item "Anaerobic Cycle"
Do	Display
Do	Displays numbers of cycles selected
↑,↓	Until item "mod.:"
Do	In the next steps the number of anaerobic cycles is modified
↑,↓	until the required number of cycles is displayed on screen
Do	number of cycles is selected and saved
Esc	Operational mode

Once the operational mode is entered again, the standard anaerobic program will perform the new selected number of evacuation and replacement cycles.

This example is useful to experiment with the Anoxomat programming display. However, we advise to leave the standard anaerobic recipe unchanged and to program a recipe as described in further on.

Changing the display intensity

The intensity of the characters on the liquid crystal display can be changed via the programming panel. In contrast to the other activities described in this chapter, this is an operational mode action. This means that you do not tip the Esc touch switch to enter the programming mode.

Table 7: Change the intensity of the display.

Touch switch	Operation
Multiple ↓	increase display intensity
Multiple ↑	decrease display intensity

The intensity of the display increases/decreases step by step with every push on the corresponding button.

Changing the pumptime jar

To check whether a jar is connected to the respective jar tube, the Anoxomat will pump on each tube a few seconds, thereby sensing the presence or absence of a jar on the tube. Normally this pumptime jar is set on 0.8 seconds.
When using very big (or thirdparty) jars, these 0.8 seconds may not be enough to sense the presence of the jar. This may also be the case when the pump gets old. The customer can easily check and change this feature.

Table 8: Change the pumptime jar

Esc	Programming mode
↑,↓	Till “menu”
Do	Select “menu”
↑,↓	Till “Program settings”
Do	Select “Program settings”
↑,↓	Till “Pump time jar se”
Do	Select “Pump time jar se”
↑,↓	Till “Mod.:”
Do	Select item “Mod.:”
↑,↓	Till first digit
Do	Select first digit
↑,↓	Till second digit
Do	Select second digit
↑,↓	Till third digit
Do	Select third digit
Do	
↑,↓	Till “Back”
Do	Select “Back”
↑,↓	Till “Back”
Do	Select “Back”
↑,↓	Till “Program system”
Do	Select “Program system”
↑,↓	Till “Save”
Do	Select item “Save”
	On the display “Are you Sure”
Do	Settings are being saved
Esc	Operational mode

Run a recipe

With the Anoxomat systems there are 2 standard recipes buttons, one for the anaerobic atmosphere and the other for a micro-aerophilic atmosphere in the jar.
When one of these buttons is pushed in the operational mode, the actions described in the standard recipe are performed. A user-defined recipe is activated with the DO button from the operational mode.

Summarising:

Anaerobic Cycle	standard anaerobic recipe
Microaerophilic Cycle	standard micro-aerobic recipe
Do	default user-defined recipe

Selection of a user defined recipe

Recipe selection is done in the programming mode, on menu level “Select recipe”.
In the next table an example with the detailed actions to select recipe 17 is shown.

Table 9: Select recipe 17

Esc	Program mode
↑,↓	Until “select recipe”
Do	Enter new menu level
↑	Until “Mod:?” is on display
Do	Select menu item, “Mod:0”
↑	Until “Mod:1” is on display
Do	Select menu item, “Mod:1?” on display
↑	Until “Mod:17” is on display
Do	Select menu item, “Mod:17?” on display
Do	Confirm
Esc	Operational mode

Pushing the "Do" button in the operational mode will execute recipe 17.

Caution: Recipe 17 becomes default and remains default until a different recipe is selected.

Programming a recipe

Procedure

In this section the activities are described which have to be done to add a recipe to, or replace a recipe in the Anoxomat system database.

Next list summarizes the actions, which should be performed subsequently:

- definition of the gas composition
- study the recipe parameters
- determine how the defined gas composition can be made
- construct recipe table
- calculation of the setpoints
- program description and programming

- select user defined recipe
- set correction mode on
- run the recipe in the correction mode
- repeat last 2 steps two to three times

In the next paragraphs the programming of a recipe on a WS9000 system is described. Gas 1 is Anaerobic gas mixture (not used in this example)
The two other gasses are separately connected to the WS9000.

Definition of the gas composition

Consider the establishment of a low oxygen concentration in a jar.
The gas composition required could be:

5% O_2
10% CO_2
85% N_2

Recipe parameters

A recipe consists of a sequence of actions each with the attributes:

Table 10: Parameters

Parameter	Description	Input
Action number	The action number defines when the action is performed in the sequence. (the lines of the recipe)	Actions are numbered from 1 to 25
Mod. Action	Which action has to be taken?	0 = no action 1 = evacuation 2 = replacement 3 = vacuumleaktest 4 = catalyst test positive (anaerobic) 5 = catalyst test negative (micro-aerophilic)
Mod. Gas	Which gas has to be used?	0 = no gas 1 = anaerobic gas mixture 2 = CO_2 [for Anoxomat models WS8080 and WS9000] 3 = N_2 [only for WS9000]
Mod. Setpoint	Until which pressure has to be evacuated or replaced? (digits xxxx in mbar pressure)	evacuate to setpoint or replace to setpoint xxxx

With the parameters named above we have to construct the recipe. In total 40 recipes can be programmed by the user. The maximum number of action lines for one recipe is 25.

Construction of a recipe table

Table 11: Example of a recipe table

Action No. (Recipe line)	Mod. Action (Which action has to be taken?)	Mod. Gas (Which gas has to be used?)	Mod. Setpoint (Until which pressure has to be evacuated or replaced?)
1	0 to 5	1 to 3	200 to 1000
2			
3			
4			
5			
-			
25			

Initially the atmospheric air in the jar has to be analysed in order to determine the exact gas composition created by a certain recipe.

At first the atmosphere in the jar is the same as the atmosphere in the laboratory environment. It is assumed that it is composed of 80% N_2 and 20% O_2.

The requested low oxygen concentration recipe can be made in one cycle by evacuating the air (reduced oxygen tension) and then replace it with the anaerobic gas mixture.

Table 12: Running a low oxygen tension recipe

Action	Mod. Action	Mod. Gas	Mod. Setpoint[I]	descriptions
1	1	0	Low set point	evacuate
2	3	0	0	vacuum leak test
3	2	3	Intermediate set point	replace with N_2
4	2	2	High set point (1000)	replace with CO_2
5	0	0	0	end of recipe

I. The setpoints have to be given in mbar of the atmospheric pressure

Calculation of the setpoints

The oxygen concentration in the air is initially 20% and should be reduced to 5%, a factor 4 times as low, in the evacuation cycle:

$$\text{Low set point} = \frac{5}{20} \times 1000 = 250$$

There has to be 10% CO_2 in the gas composition. During a range of 100 mbar CO_2 has to be injected:

$$CO_2 \text{ dosing range} = \frac{1000}{10} = 100$$

High set point = 1000

Intermediate set point = High set point – dosing range CO_2 (1000 – 100) = 900

For accuracy reasons it is better to have the long ranges in the deepest vacuum points. The explanation is straightforward. Injecting gas means physically that a valve is opened and is closed again as soon as a certain pressure setpoint is reached. If there is a deep vacuum in the jar gas is injected into the jar with a high speed. The lower the vacuum in the jar the easier the gas flow can be better controlled and the more accurate the end result will be.

Program description and programming

Similar to the previous program activities the recipe table has to be programmed.

Table 13: Programming steps for recipe 17 with 5% O_2, 10% CO_2 and 85% N_2

Esc	Program level	Item	input
↑,↓ /do	<Program recipe>	<recipe number>	<17>
↑,↓ /do	<Program recipe>	<action number>	<1>
↑,↓ /do	<Program recipe>	<mod. Action>	<1>
↑,↓ /do	<Program recipe>	<mod. gas>	<0>
↑,↓ /do	<Program recipe>	<mod. Setpoint>	<250>
↑,↓ /do	<Program recipe>	<action number>	<2>
↑,↓ /do	<Program recipe>	<mod. Action>	<3>
↑,↓ /do	<Program recipe>	<mod. gas>	<0>
↑,↓ /do	<Program recipe>	<mod. Setpoint>	<0>
↑,↓ /do	<Program recipe>	<action number>	<3>
↑,↓ /do	<Program recipe>	<mod. Action>	<2>
↑,↓ /do	<Program recipe>	<mod. gas>	<1>
↑,↓ /do	<Program recipe>	<mod. Setpoint>	<900>
↑,↓ /do	<Program recipe>	<action number>	<4>
↑,↓ /do	<Program recipe>	<mod. Action>	<2>
↑,↓ /do	<Program recipe>	<mod. gas>	<1>
↑,↓ /do	<Program recipe>	<mod. Setpoint>	<1000>
↑,↓ /do	<Program recipe>	<action number>	<5>
↑,↓ /do	<Program recipe>	<mod. Action>	<0>
↑,↓ /do	<Program recipe>	<mod. gas>	<0>
↑,↓ /do	<Program recipe>	<mod. Setpoint>	<0>
Esc	Operational mode		

Correction Mode

Before a newly programmed recipe can be used it has to be executed with the Anoxomat system in the correction mode. The Anoxomat system calculates the settings for the new defined program.

Caution: When a new-programmed recipe is not executed in the correction mode the expected composition will differ from the real one!

The programming steps are:

Table 14: Correction mode

Esc	program mode
↑,↓ /Do	<correction mode> <on>
Esc	operational mode
Do	start recipe 17 in correction mode

After the recipe is executed in the correction mode the correction mode returns automatically to the "off" state. This is done to prevent the customer from running a recipe in the correction mode always.

In certain cases, when setpoints lie close to each other, running the recipe in the correction mode one or two times more can increase the accuracy.

Caution: Recipe 17 can be run with or without a leaktest. This depends on whether the quality assurance is switched on or not.

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Programming a recipe

Table 15: Programming a recipe

Esc	"PROGRAM MODE"
↑	until "Menu"
Do	Select "Menu"
↑	until "Program Recipe"
Do	Select "Program Recipe"
↑	until "Recipe number"
Do	on display "Recipe?"
↑	until right number
Do	on display "Recipe number"
↑	until "**Action number**"
Do	on display "Action?"
↑	until right Action number
Do	on display "Action number"
↑	until **"Mod. action"**
Do	on display "Mod.?"
↑	until right Action
Do	on display "Mod. action"
↑	until "**Mod. gas no**"
Do	on display "Mod?"
↑	until right gas selection
Do	on display "Mod. gas no"
↑	until "**Mod. setpoint**"
Do	on display "Mod.?"
↑	until right setpoint number
Do	on display "Mod. setpoint"
↑	until "Read action"
Do	on display: "current" Recipe number - Action number Mod action number : Gas number : setpoint
Do	on display "Read action" (With esc : exit "PROGRAM MODE")
↑	until "Back"
Do	on display "Program recipe"
↑	until "Back"
Do	on display "Menu" esc : exit "PROGRAM MODE"

Mart Jar system

Jar sizes

Table 16: Specifications of Mart anaerobic jars

Characteristic / size in mm.	AJ9023	AJ9026	AJ9027	AJ9028
Inner height (including catalyst height)	240	240	240	240
Outer height (including clamp and coupling)	320	320	320	320
Inner diameter	123	140	190	230
Outer diameter (excluding clamp)	154	170	220	260
Volume	2.9Lt	3.8Lt	6.9Lt	10.3Lt
Rack for 12 petridishes, 9-10 cm	1	1	2	3
Rack for 18 petridishes, 6cm		3		
Catalysts	1	1	2	3

Jar Material

Jar and Lid material: Transparent PMMA (Poly Methyl Meth Acrylic or Cast Acrylic)
The extremely solid, durable construction guarantees a long life
The Mart jarlid clamp construction meets the highest quality requirements, mechanical as well as microbiological

The Mart Jars are never to be cleaned with alcohol, and not to be cleaned under temperatures of more than 80ºC. Please view under “Jar cleaner and disinfectant”

Jar spare parts

All jar parts are supplied as spare parts
- Jar
- Lid
- Rubber O-ring
- Catalyst clip(s)
- Stainless steel jar clamp with white coating
- Snap shut coupling for Anoxomat

Jar cleaner and disinfectant

The Mart Jars are never to be cleaned with alcohol, or to be put in the autoclave.

Mart Microbiology B.V. advises Halamid Jar cleaner and disinfectant
- Available in 2.5 (500 litres) or 5 kg (1.000 litres) buckets
- Based on latent chlorine and oxygen
- Effective against bacteria, fungi and viruses.
- Solution 0.5%

Jar maintenance

To be certain that the jars are maintained correctly and regularly, a small box with necessities to maintain all jars working with the Anoxomat (original Mart jars, as well as modified jars of other brands) is available
This kit includes 10 replacement snap shut couplings (these cannot be cleaned, only be greased with Teflon spray), tools, cleanser and lubricant, complete with instructions.
Also available is a refill set consisting of only the used materials of the toolkit

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